Koichiro MIURA*: *Amoebophilus dangeardii*, a new Zygomycete parasitic on Amoebae

三浦宏一郎*: アメーバ寄生性の接合菌類の1新種 *Amoebophilus dangeardii*

In continuation of studies on fungi destructive to microscopic animals, a fungus ectoparasite of amoebae is herein described as a new species. The fungus appeared abundantly in a maize-meal-agar plate inoculated with a small pinch of moss cushion which had been kindly collected by Dr. S. Tokumasu in woods near Danto, Kumamoto Pref. on November 5, 1968. It occurred on *Amoeba* sp. in the form of a bush-like tuft of short filaments.

Structure & Reproduction The attack of the fungus parasite in question begins apparently with the adhesion of one or more of its conidia to the pellicle of the host animal. Each of the adhering conidia puts forth somewhat obliquely a delicate germ tube mostly from a position near one of its ends into the host protoplasm. When the germ tube has attained a length of about 5 μ, it widens abruptly to form a globose or bilobate to tetralobate haustorium. With a supply of nutriment from the host, the anchored conidium becomes somewhat thick and robust. Then, it puts forth 2-5 fine cylindrical outgrowths from parabasal positions opposite the haustorium and, often, from the apical position. After attaining a length of 2-12 μ, the outgrowths widen rather abruptly and elongate to form clavate or cylindrical branchlets with rounded apex. The branchlets (the primary branchlets) are constricted at the base but not delimited by a septum. One (occasionally two) of these primary branchlets gives rise to a new outgrowth from its free end. This outgrowth develops into a secondary branchlet, following the same manner as was described above for the primary branchlet. The secondary branchlet may form the tertiary one. The mature thallus, thus established, consists of an affixed swollen conidium and several branchlets. It is coenocytic, branched and segmented; each segment of the thallus (the affixed conidium or each branchlet) is clavate or cylindrical or fusiform,

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up to 30 μ long and 2.8-4.0 μ wide and connected by a narrow isthmus to the segment from which it has arisen. The thallus is procumbent by the whole length on the surface of the substratum.

Asexual reproduction is by means of true conidia produced in chains. A conidial chain arises mostly from one of terminal branchlets of a thallus (usually from the extreme end of a thallus). It develops on the upwardly curved terminal portion of the fertile branchlet or on the up-turned terminal elongation of the fertile branchlet. Conidia are formed in acropetal succession after such a manner as was observed in the branchlet formation of the thallus. The chain of conidia is aerial and usually simple, and easily broken up on slight disturbance. The detached conidium is acerose or slenderly fusiform, 19-30 μ long and 1.9-2.9 μ wide.

Sexual reproduction of the present fungus is by a process of conjugation of zygophoric hyphae. Zygophoric hyphae to mate with each other arise from a single thallus (homothallic) and they are heteromorphic. One of the zygophoric hyphae grows out of a terminal end or of a terminal prolongation of a thalline branchlet. The hypha is clavate and constricted at the base and looks like an undersized thalline branchlet. The other arises from a position just below the clavate one; it is cylindrical, curved and not constricted at the base. The cylindrical hypha elongates somewhat circuitously and, often, more or less circularly around the lower portion of its clavate partner. Then, the tip of the cylindrical hypha comes into contact with the clavate one at a position just above the constriction. An intermediate portion of the cylindrical hypha swells up to form a globose body, a young zygospore. The mature zygospore is polyhedral and 5.7-9.0 μ in diameter, containing a spherical protoplast. Germination of a zygospore has not been observed.

The protoplasmic contents of the infected animal are gradually consumed by the parasite. The wrinkled pellicle of the host animal shrinks into an inconspicuous mass together with the emptied thallus of the parasite in later stages. The young thallus of the present fungus seems to be able to capture an additional amoeba through adhesion to the primary branchlet, although such a predacious habit has ever been displayed by only two individuals. The thalline branchlets do not seem to act as dispersal units. Therefore, the present fungus may be said to have an ecological feature intermediate

between ectoparasitic and predacious.

Taxonomic consideration The genus *Amoebophilus* was erected by Dangeard (1910) based on *A. penardii* Dang. (originally as *A. penardi*) which was found by Dangeard himself on an amoeba, *Pelomyxa vorax* Dang. At the same time, two additional binominals, *A. korotneffii* Dang. and *A. caudatus* Dang., were proposed by him; *A. korotneffii* was given to 'la queue' of *Longicauda amoebina* which had been originally described by Korotneff (1879/1880) as a caudate amoeba, and *A. caudatus* was given to the fungus parasites found by Penard (1902) on *Amoeba nobilis* Penard. Dangeard said, 'Nous donnons à celle que nous avons étudiée sur le *Pelomyxa* le nom d'*Amoebophilus Penardi*; provisoirement, afin d'éviter la création d'autres noms de genre, on pourra désigner celle de Korotneff sous le nom d'*Amoebophilus Korotneffii* sp. nov.; la troisième espèce décrite par Penard à la suite de Leidy sera l'*Amoebophilus caudatus*.' The fourth species was described by Drechsler (1959) as an ectoparasite of *Amoeba guttula* Dujardin under a scientific name of *Amoebophilus sicyosporus* Drechsler.

'Les filaments de l'*Amoebophilus penardii*' are coenocytic, unbranched, cylindrical and more or less narrowed at the base. Dangeard (1910) observed neither adhesive disc nor haustorium at the place where the filament was affixed to the host pellicle. He observed the apical bud formation of the filament and thought 'cette cellule (la cellule provenant de ce bourgeonnement) peut se détacher à cet état et constitue une spore qui reproduira l'organisme'. But he found that some buds elongated and formed individuals consisting of two segments of nearly equal length connected by a very small isthmus and that in some of these segmented individuals an additional bud was formed from the terminal segment.

According to Korotneff (1879/1880), 'la queue de l'animal' (the tuft of filaments of *A. korotneffii*) is triple; its three parts are completely independent and have a form of the antler of a stag. Korotneff observed that the protoplasmic contents of terminal branches became converted into series of bodies presenting a refringent appearance. These refringent bodies were later regarded as spores by Dangeard (1910). The filaments of *A. caudatus* are tubular and branched; each filament has a protuberance-like or horn-shaped basal part. Near the basal part of each filament, Penard (1902) noticed the presence of a small colorless body bearing 2-3 distally lobed

branches in stellate or trefoiled arrangement. He considered the lobed body was 'le commencement de la germination d'une spore'. Penard did not make any mention of detached spores. But he too observed refringent bodies in the filament.

The attack of *A. sicyosporus* begins with adhesion of a conidium of cylindrical shape somewhat like that of cucumber fruits to the pellicle of the host. The affixed conidium intrudes a haustorium of globose or bilobate or trilobate shape from one of its ends and gives rise to new conidia singly or in chains from positions near the affixed end and at the free end. The thallus of this fungus is merely an affixed conidium equipped with a haustorium. Sexual reproduction is by a process of conjugation of the zygophoric hyphae originating from separate thalli and results in the formation of a zygospore. The zygospore is pronouncedly polyhedral at maturity.

Members of the genus *Amoebophilus*, excepting *A. sicyosporus*, have been described very briefly and sketchily and the genus has been characterized only by the short filaments growing on rhizopods. Therefore, a short discussion is herein indispensable concerning development and reproduction of *Amoebophilus* species hitherto described (especially of *A. penardii*, the type species).

In the case of *A. sicyosporus* and the present fungus, the attack of the parasite begins with the adhesion of the conidia to the outer surface of the host animal. In both fungi the adhering conidium develops into a segmented 'individual' by budding. The 'individual' of *A. sicyosporus* consists of an affixed conidium and its progenies (conidia); the thallus is an affixed conidium itself. In contrast, the 'individual' of the present fungus consists of an affixed conidium and its branchlets; the thallus is branched and segmented. In the remaining species, it is not known how the attack of the parasite starts. In *A. penardii*, the segmented individual results through the apical bud formation. Dangeard (1910) regarded the bud as a spore. If Dangeard's view is correct, the 'individual' of *A. penardii* consists of a non-segmented thallus and a spore (or a short spore chain). Dangeard observed some buds elongated to form individuals consisting of two segments of nearly equal length. Judging from size and shape of the 'mature' spore, the thallus (the basal segment) of *A. penardii* is probably an affixed spore itself. Accordingly, *A. penardii* is not essentially different from *A. sicyo-*

sporus as regards structural make-up of the thallus and the process of asexual reproduction. On the other hand, as Dangeard did not make any mention of detached spores, it is not impossible to say that the bud observed by Dangeard may be a branchlet of the thallus. If this view is true, *A. penardii* has such a segmented thallus as the present fungus has. And, conidia of *A. penardii* are probably formed on the branchlet(s). As a matter of course, it should be herein taken into consideration that *A. penardii* may reproduce by zoospores or aplanospores. According to Dangeard, however, the filament of *A. penardii* contains 1-8 nuclei, which are as wide as the filament. Judging from number and size of the nuclei, *A. penardii* is unlikely to be a mastigomycete. Judging from structural features of the thallus, *A. penardii* is unlikely to be an aplanospore-producing fungus, such as a mucoralean fungus or a trichomycete. Accordingly, it is likely that *A. penardii* reproduces asexually by conidia produced in such a manner as is observed in the present fungus. Whichever view may be justified, the basic mode of development of *A. penardii* is budding. And, budding is the process by which the 'individuals' of *A. sicyosporus* and the present fungus develop. In contrast with the fungi above discussed, *A. korotneffii* and *A. caudatus* have the filaments lacking pronounced constrictions associated with the budding process.

Amoebophilus sicyosporus has a globose or bilobate or trilobate haustorium. The present fungus has the same type of haustorium. In *A. penardii*, any organs for attachment were not observed. But Dangeard admitted that the manner of attachment needed further study. It seems rather reasonable to assume the presence of a haustorium, probably of the lobate type, in *A. penardii*. 'Le commencement de la germination d'une spore' of *A. caudatus* is morphologically very similar to the haustorium of *A. sicyosporus* or of the present fungus.

Sexual reproduction is known only in *A. sicyosporus* and the present fungus. Both fungi resemble each other as regards the manner of sexual reproduction and morphological features of the zygospore, although *A. sicyosporus* is heterothallic and the present fungus is homothallic. In *A. korotneffii* and *A. caudatus*, refringent bodies developed in the terminal branches of the filaments. Those of *A. korotneffii* shown in the figure given by Korotneff (1879/1880, Pl. XXXV, Fig. 3) present some resembrance to the

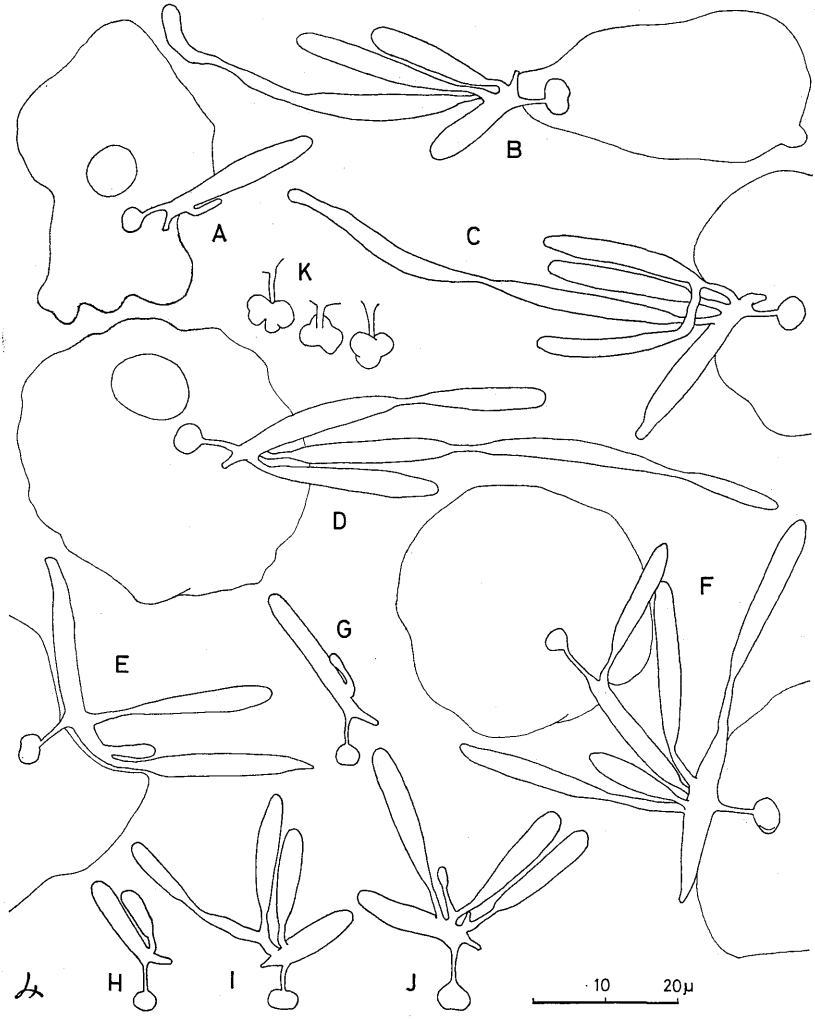

Fig. 1. *Amoebophilus dangeardii* Miura. A-F. Immature thalli growing on amoebae. G-J. Immature thalli. K. Well-developed haustoria.

uninucleate sporangiospores observed in the Trichomycetes (Lichtwardt, 1973). Those of *A. caudatus* figured by Penard (1902, p. 66, Fig. 6) are suggestive of the oil-drops observed by Leidy (1879, Pl. IX, Fig. 12) in caudal filaments of *Ouramoeba vorax* Leidy (? an amoeba bearing a fungus parasite).

In conclusion, the present author thinks that the present fungus should be treated as a member of the genus *Amoebophilus*, until the correct interpretation of the segmented 'individual' of *A. penardii* is made. The present fungus is clearly distinguished from other species of *Amoebophilus* so far described by having a branched thallus and an aerial chain of conidia and by showing homothallism in zygospore formation. The *Amoebophilus* seems to belong to the Zoopagales (Zygomycetes).

Incidentally, it may be worth-while to make mention of two unnamed fungi which show close resemblance to the present fungus especially in structural make-up of the filaments. One of them is the caudal appendages of *Ouramoeba botulicauda* Leidy, which was erroneously described as a caudate amoeba by Leidy (1879). The appendages are tufted moniliform filaments consisting of series of elongate elliptical bodies resembling strings of sausages. The other is 'büschelig gehäufte Fäden' found by Geitler (1937) on *Amoeba proteus*. The 'Fäden' are constricted at the base and also at intermediate positions at intervals and connected with a irregularly lobed haustorium by a slender filament. In neither fungi, any dispersal units were reported. But polyhedral 'Dauerzellen' were observed in the latter fungus. 'Die Dauerzellen' are manifestly azygospores. The present fungus shows more resemblance to these two fungi than to any species of *Amoebophilus*. But it is distinguished from the caudal appendages of *O. botulicauda* by having oversized, cylindrical to clavate segments and from Geitler's fungus by forming zygospores instead of azygospores.

Amoebophilus dangeardii Miura, sp. nov.

Fungus ectoparasiticus sine hyphis mycelialibus filamentosis. Thallus incoloratus, coenocyticus, ramosus, segmentatus, ad extremitatem conidia catenata pullulans; segmenta clavata vel cylindrica vel fusiformia, basi constricta, usque 30 μ longa, 2.8-4.0 μ lata. Conidia incolorata, unicellularia, laevia, acerosa vel fusiformia, 19-30 μ longa, 1.9-2.9 μ lata, prope unum extremum ad *Amoebam* inhaerentia, ibi haustorium in animal intrudentia, tum moderate tumescentia, mox prope extremum affixum et saepe ex altero extremo 2-5 ramulos primarios pullulantia, deinde ex 1-2 ramulos primarios unum ramulum secundarium et saepe ex uno ramulo secundario unum ramulum tertiarium pullulantia, ita in thallos segmentatos crescentia. Haustoria globosa vel biloba—tetraloba, 2.3-3.8 μ lata, pedicello 3.3-6.4 μ longo et 0.4-

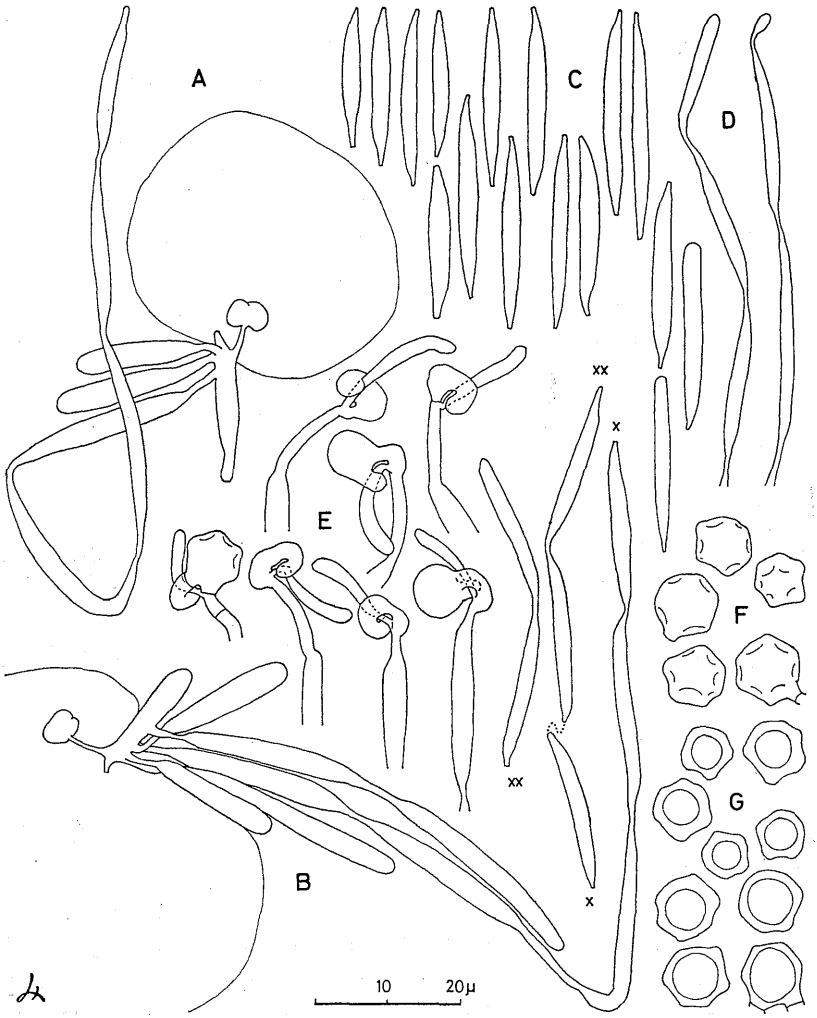

Fig. 2. *Amoebophilus dangeardii* Miura. A–B. Mature thalli bearing a conidial chain. C. Conidia. D. Terminal portions of conidial chains. E. Zygospore formation. F. Mature zygospores (surface view). G. Mature zygospores (optical section).

0.7 μ lato praedita. Hyphae zygosporiferae heteromorphae; una clavata 10–18 μ longa, 1.7–3.0 μ lata; altera cylindrica 1.8–2.6 μ lata. Zygosporae ex hypha cylindrica oriundae, hyalinae, primo globosae, in maturitate multan-

gulae, 5.7-9.0 μ in diametro, cellulam viventem globosam circumdantes. 'Homothallic'.

Amoebas (*Amoeba* sp.) 25-70 μ latas enecans habitat in coloniis muscorum prope Danto, Kumamoto (November, 1968).

Typus (Iconotypus): Figura 2-B.

The specific epithet is in honor of P. A. Dangeard, the author of the genus *Amoebophilus*.

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アメーバ (*Amoeba* sp.) 上に外部寄生をする一接合菌 **Amoebophilus dangeardii** Miura (新種) を報告する。本種はその分生子がアメーバ外皮に粘着することにより感染を始める。次いで，アメーバ内に吸器を，アメーバ体表上に分枝・分節した短い菌体を発達させる。無性生殖は気生の鎖状に連鎖した分生子により，有性性殖は接合胞子形成による。本種の分類学的考察にあたり，従来不明確であった *Amoebophilus* 属の形質を検討した。本属は明らかにトリモチカビ目 Zoopagales (接合菌類) に所属すると思われる。

正 誤 (Errata)

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